Journal of Health Science, **52**(2) 118–123 (2006)

L-Cysteine Influx and Efflux in Human Erythrocytes: the Role of Red Blood Cells in Redox and Metabolite Homeostasis in the Plasma

Deniz Yildiz,* Buse Hilal Ates, Ceylan Uslu, and Haydar Oztas[1]

Biology Department, Faculty of Science and Arts, Mustafa Kemal University, Antakaya, Hatay, Turkey

(Received October 13, 2005; Accepted November 26, 2005)

The objective of this study was to investigate L-cysteine influx and efflux in human erythrocytes. L-cysteine is an amino acid required for glutathione synthesis in erythrocytes. In addition to being incorporated into glutathione, the soluble antioxidant L-cysteine plays a role in the maintenance of a proper intracellular or extracellular redox status. Recent investigations have pointed out that L-cysteine may contribute to redox homeostasis in the plasma and in the periplasm of some bacteria. Thus L-cysteine availability in the plasma may influence the oxidized/reduced state of several other metabolites normally found in the plasma. Our L-cysteine uptake studies demonstrated that erythrocytes can respond to an increase in the L-cysteine concentration in the extracellular media and influx L-cysteine in a concentration dependent-manner. The L-cysteine efflux is also time and concentration dependent. Erythrocytes pretreated with higher concentration of L-cysteine displayed higher efflux rates. Erythrocytes pretreated with L-cysteine 1 mM displayed efflux and increased the free-SH concentrations up to 0.184 ± 0.010 mM in the incubation media in 1 hr. While this concentration reached 0.843 ± 0.012 mM in 10 mM-L-cysteine pretreated erythrocytes. Our results also showed that the L-cysteine efflux is partly mediated by the Alanine-Serine-Cysteine (ASC) system. The presence of alanine or serine in the incubation media decreased the rate of efflux by about 16%. Our results also showed that the L-cysteine efflux process is not a simple diffusion but a carrier-mediated process. When compared with N-acetyl-L-cysteine (NAC), which is known to diffuse through the membranes, L-cysteine displayed a higher efflux rate under the same conditions. Pretreatment of erythrocytes with L-cysteine 4 mM increased the free-SH concentration to 0.48 ± 0.005 mM whereas the same concentration of NAC brought the free-SH concentration to 0.36 ± 0.01 mM in the incubation media. Our results suggest that erythrocytes may contribute to redox and metabolite homeostasis of the plasma.

Key words —— erythrocytes, cysteine efflux, redox homeostasis

INTRODUCTION

L-cysteine is an important free-SH containing amino acid. In addition to being incorporated into proteins, L-cysteine is also utilized in the synthesis of an important tripeptide, glutathione (GSH).[1] Erythrocytes lack a functional protein synthesizing system. Thus erythrocytes can not incorporate the influxed L-cysteine into proteins. However, L-cysteine is required for erythrocyte integrity. L-cysteine is mainly utilized in glutathione synthesis in erythrocytes. GSH is a tripeptide composed of glutamic acid, cysteine, and glycine. Among these three amino acids, L-cysteine availability determines the rate of GSH synthesis.[1] The functional-SH group of GSH is also provided by the amino acid L-cysteine. GSH functions as a soluble antioxidant and protects cells against free radicals and lipid peroxidation.[2,3] It is also involved in detoxification of several xenobiotics.[3–6] GSH reacts with toxic xenobiotics in a glutathione S-transferase-catalyzed reaction and forms glutathione conjugates that are then transported out of the cells in an ATP-dependent manner.[7–11] Although GSH is actively synthesized in erythrocytes, the synthesized GSH is not effluxed from the erythrocytes.[12] Thus, in contrast to the liver, erythrocytes do not provide the plasma with GSH.[13]

In this study we investigated whether erythrocytes provide the blood plasma, and therefore other

[1]Present address: Biology Education Department, Faculty of Education, Selcuk University, Konya, Turkey
*To whom correspondence should be addressed: Biology Department, Faculty of Science and Arts, Mustafa Kemal University, Antakaya, Hatay, Turkey. Tel.: +90-326-2673436; Fax: +90-326-2455867; E-mail: dyildiz@mku.edu.tr

cells with L-cysteine which is the rate-limiting amino acid in GSH synthesis. The study also examined whether erythrocytes function in regulation the of L-cysteine concentrations in the plasma *via* uptake when its concentration is raised and carrying and releasing it at distal tissues where its concentration is relatively lower. L-cysteine influx into erythrocytes has been extensively studied. It has been shown that the L-cysteine transport into erythrocytes and other cells is mainly mediated by Na-dependent and Na-independent systems.[14] However, to the best of our knowledge, L-cysteine efflux from erythrocytes has not been studied in detail.

MATERIALS AND METHODS

Materials ——— N-acetyl-L-cysteine (NAC), L-cysteine, L-alanine, and L-serine were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). 5,5′-Dithiobis(-nitrobenzoate) (DTNB) was obtained from Fluka BioChemica, (Switzerland). Blood was obtained from the blood bank of Mustafa Kemal University Research Hospital as 300-ml units derived from human donors with no prerecorded medical conditions.

Preparation of Erythrocytes ——— Plasma was separated by centrifugation at 2000 *g* for 5 min. The plasma and the buffy coat were then removed and discarded. The resulting erythrocyte pellet was washed twice with two volumes of phosphate-buffered saline (PBS) (nine parts of NaCl 0.15 mM and one part of potassium phosphate buffer 0.1 M, pH 7.4) and was further used in the experiments at 20% hematocrit.[15] PBS-glucose contained glucose 8 mM in PBS.

L-Cysteine Uptake Studies ——— Washed erythrocytes 0.25 ml were suspended in 1 ml of PBS-glucose containing 1, 4, and 10 mM concentrations of L-cysteine and incubated for 15, 30, 60, and 90 min at 37°C in a water bath. At the end of incubation, the erythrocytes were removed, centrifuged, and the supernatants were discarded. The free-SH concentrations in erythrocytes were then determined as described by Sedlak and Lindsay.[16] Briefly 100 μl of erythrocytes were lysed in 100 ml of 10% tricarboxylic acid (TCA) prepared in sodium phosphate-EDTA buffer (sodium phosphate 0.01 M/EDTA 0.005 M). The erythrocyte lysates were then centrifuged at 12000 *g* for 5 min. At the end of centrifugation, 100 μl of the supernatant was mixed with 1.9 ml of Tris-EDTA buffer containing DTNB 0.6 μM/ml (Tris base 262 mM, EDTA 13 mM, pH 8.9). Samples were allowed to stand for 5 min to develop color. The absorbance of samples were then measured at 412 nm and the concentrations of free-SH were calculated using the mM extinction coefficient of 13.6.

L-Cysteine Efflux Studies ——— Washed erythrocytes 0.25 ml were resuspended in 1 m of PBS-glucose in the presence of different concentrations of L-cysteine. Erythrocytes were incubated at 37°C in a water bath for 1 hr to allow the uptake process. At the end of incubation, the erythrocytes were centrifuged and the supernatants were discarded. The erythrocytes were then resuspended in 1 ml of fresh PBS-glucose or without glucose, containing either NaF, alanine, or serine and incubated at 37°C for the indicated times to allow the efflux process. At the end of incubation the erythrocytes were centrifuged and the supernatants were transferred to fresh tubes. The free-SH concentrations in the supernatant was then measured as described above.

Statistical Analysis ——— One-way analysis of variance (ANOVA) and Student-Newman-Keuls multiple comparison tests were applied to process the data statistically. All tests were performed on triplicate samples. Results are expressed as mean ± S.D. A p-value of < 0.05 as considered statiscally significant.

RESULTS

Table 1 shows the results of the time- and concentration-dependent L-cysteine uptake studies. Erythrocytes accumulated L-cysteine from the media efficiently. The uptake process increased with increasing concentration of L-cysteine. The uptake increased for 1 hr and further incubation did not increase the uptake of L-cysteine. The free-SH concentration in erythrocytes doubled in 1 hr when treated with L-cysteine 4 mM. Free-SH concentrations increased to 3.1-fold the control levels in 1 hr in L-cysteine 10 mM treated erythrocytes. Table 2 shows the results of the L-cysteine efflux studies. Our results demonstrated that erythrocytes may transport the influxed L-cysteine back into the media from the cytoplasm when L-cysteine concentration decreases. The rate of the L-cysteine efflux was dependent on time and the intracellular levels of L-cysteine. The incubation of L-cysteine-pretreated erythrocytes effluxed the amino acid in an increasing manner for up to 1 hr, and further incubation did

Table 1. Time Course L-Cysteine Uptake by Erythrocytes

	Free-SH concentrations in erythrocytes (mM)			
	15 min	30 min	60 min	90 min
Control	1.409 ± 0.057	1.409 ± 0.025	1.412 ± 0.042	1.429 ± 0.023
Cysteine 1 mM	1.407 ± 0.036	$1.723 \pm 0.024^{a)}$	$1.735 \pm 0.096^{a)}$	$1.723 \pm 0.013^{a)}$
Cysteine 4 mM	$2.312 \pm 0.042^{a)}$	$2.582 \pm 0.074^{a)}$	$2.785 \pm 0.084^{a)}$	$2.772 \pm 0.024^{a)}$
Cysteine 10 mM	$3.379 \pm 0.075^{a)}$	$4.258 \pm 0.040^{a)}$	$4.382 \pm 0.033^{a)}$	$4.447 \pm 0.162^{a)}$

Washed erythrocytes were resuspended in PBS-glucose containing the indicated concentrations of L-cysteine and incubated for 15, 30, 60, and 90 min. At the end of incubation free-SH concentration in the erythrocytes were determined. Results are the mean ± S.D. of three separate experiments. *a*) Significantly different from the control. $p < 0.05$.

Table 2. Time Course of L-Cysteine Efflux from Erythrocytes Preincubated with L-Cysteine

	Free-SH concentrations in the incubation medium (mM)		
	30 min	60 min	90 min
Control	0.00	0.00	0.00
Cysteine 1 mM	$0.0823 \pm 0.013^{a)}$	$0.184 \pm 0.010^{a)}$	$0.182 \pm 0.006^{a)}$
Cysteine 4 mM	$0.291 \pm 0.060^{a)}$	$0.462 \pm 0.020^{a)}$	$0.478 \pm 0.018^{a)}$
Cysteine 10 mM	$0.621 \pm 0.012^{a)}$	$0.843 \pm 0.012^{a)}$	$0.873 \pm 0.065^{a)}$

Washed erythrocytes 0.25 ml were first preincubated in 1 ml of PBS glucose containing L-cysteine 1, 4, and 10 mM for 1 hr. At the end of incubation erythrocytes were centrifuged, the supernatants were discarded and the resulting erythrocyte pellets were washed. The washed erythrocytes were then resuspended in 1 ml of fresh PBS-glucose and incubated further for 30, 60, and 90 min to allow the efflux process. At the end of incubation free-SH levels in the supernatants were measured. Results are the mean ± S.D. of three separate experiments. *a*) Significantly different from the control. $p < 0.05$.

not result in additional efflux. The results shown in Fig. 1 also show that the efflux process was dependent on energy. The incubation of erythrocytes in glucose-free media or with the addition of NaF inhibited the efflux process. However, the absence of glucose or addition of NaF did not completely prevent the efflux process. This may indicate the presence of an ATP-independent efflux system. The results shown in Fig. 2 indicate that the efflux may be partly mediated by the Alanine-Serine-Cysteine (ASC) system. The addition of L-serine and L-alanine significantly inhibited the L-cysteine efflux from erythrocytes. The results shown in Fig. 3 indicate that L-cysteine NAC efflux processes are not identical. L-cysteine efflux is significantly higher than NAC efflux.

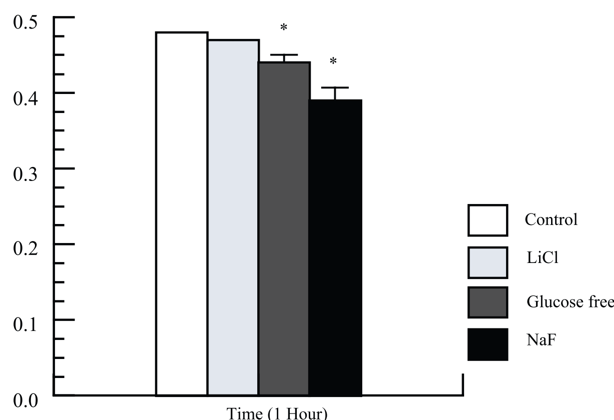

Fig. 1. Energy Dependency of L-Cysteine Efflux from Erythrocytes

Washed eryhtrocytes 0.25 ml were resuspended in 1 ml of PBS glucose containing L-cysteine 4 mM and incubated for 1 hr. At the end of incubation, erythrocytes were centrifuged, supernatants were discarded and resulting erythrocyte pellets were washed. All groups were then resuspended in 1 ml of buffer composed as described below. The control group was resuspended in PBS-glucose. The LiCl group contained LiCl in PBS instead of NaCl. The glucose free group contained PBS without glucose. The NaF group contained glucose-free PBS and NaF 10 mM. All groups were then incubated further for 1 hr and free-SH levels were determined in the supernatants. Results are the mean, S.D. of three separate experiments. *Significantly different from the control $p < 0.05$.

DISCUSSION

As shown in the Tables 1 and 2, erythrocytes take up L-cysteine from the media effectively in a time- and concentration-dependent manner. The uptake process continued to increase as the L-cysteine concentration increased. This result indicates that eryth-

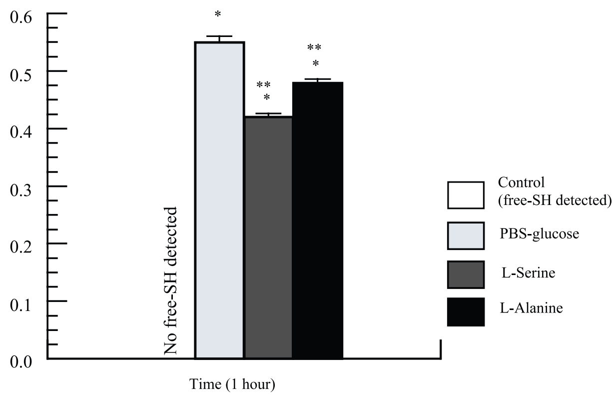

Fig. 2. Effects of Alanine and Serine on L-Cysteine Efflux

Washed erythrocytes 0.25 ml were resuspended in 1 ml of PBS-glucose containing L-cysteine 4 mM except the control group and incubated for 1 hr. At the end of incubation erythrocytes were centrifuged, the supernatants were discarded and the resulting erythrocyte pellets were washed. All groups were then resuspended in 1 ml of PBS-glucose composed as described below. The control group and the PBS-glucose group were resuspended in PBS-glucose only. The L-serine group was resuspended in PBS-glucose containing L-serine 4 mM and the L-alanine group was resuspended in PBS-glucose containing L-alanine 4 mM. All groups were then incubated further for 1 hr and free-SH levels were determined in the supernatants. Results are the mean, S.D. of three separate experiments. *Signicantly different from the control group. **Significantly different from the PBS-glucose group. $p < 0.05$.

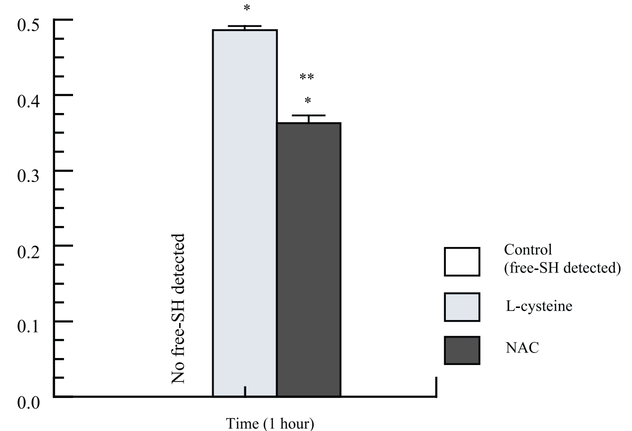

Fig. 3. Comparision of L-Cysteine and NAC Efflux from Eryhtrocytes

Washed erythrocytes 0.25 ml were incubated for 1 hr in 1 ml of PBS-glucose containing L-cysteine 4 mM or NAC 4 mM. Control group was incubated in PBS-glucose only. At the end, of incubation erythrocytes were centrifuged, the supernatants were discarded and the resulting erythrocyte pellets were washed. The washed erythrocytes were then resuspended in 1 ml PBS-glucose and incubated for 1 hr and the free-SH levels were determined in the supernatants. Results are the mean, S.D. of three separate experiment. *Significantly different from the control group. **Significantly different from the L-csteine group. $p < 0.05$.

rocytes are equipped with efficient L-cysteine uptake systems. The concentration of L-cysteine in the plasma is usually low.[17] Thus its difficult to ascribe a certain role for the presence of such an efficient uptake systems in erythrocytes. However, it could be suggested that erythrocytes are equipped to function as an emergency mechanism against L-cysteine increase in the plasma since it is known to be toxic at high concentrations.[18] This possibility also predicts that erythrocytes may function in the modulation of L-cysteine concentrations in the plasma. Erythrocytes may take up L-cysteine when its concentration is elevated, especially as a result of intake of food rich in L-cysteine, and transport and release it to distal tissues where its concentration is lower. Our results showed that erythrocytes efflux L-cysteine in a concentration-dependent manner. The efflux rate is higher in erythrocytes treated with higher concentrations of L-cysteine. The L-cysteine uptake in erythrocytes is mainly mediated by the Na- and ATP-dependent ASC system and Na-independent uptake systems.[19,20] Previous studies demonstrated that omitting Na ions from the incubation medium by using LiCl instead of NaCl inhibits L-cysteine uptake by erythrocytes.[21] It has also been shown that inhibitors of ATP synthesis such as NaF in erythrocytes decreases L-cysteine uptake by erythrocytes.[21] We investigated whether NaCl replacement with LiCl and inhibition of ATP synthesis also decrease the L-cysteine efflux process in erythrocytes. The results demonstrated that, in contrast to the uptake process, the efflux process was not dependent on the presence of Na ions. However, the incubation of erythrocytes both in the absence of glucose and NaF treatment without glucose decreased the efflux process significantly. Inhibition of the efflux process by ATP depletion suggests that the same ASC system that is involved in L-cysteine uptake may also function in L-cysteine efflux from erythrocytes. To confirm this view, we observed the changes in L-cysteine efflux in the presence of alanine and serine amino acids, the other substrates for the ASC system. The presence of alanine or serine in the media outside erythrocytes significantly inhibited the L-cysteine efflux from the erythrocytes. Thus it could be concluded that the ASC system is involved in the efflux process and alanine and serine both function as transacting inhibitors of L-cysteine efflux. However, a marked degree of efflux remains even when ATP synthesis is inhibited in erythrocytes, suggesting the presence of other systems involved in L-cysteine efflux. A significant portion of the efflux appears to be mediated by ATP-independent systems. We also investigated whether the N-acety-

lated counterpart of L-cysteine, NAC, follows a similar pattern and is exported form the erythrocytes following its uptake. Although NAC crosses membranes by simple diffusion, L-cysteine cannot cross the membranes easily. Our results showed that erythrocytes also release NAC to the extracellular media. However, L-cysteine efflux is significantly higher than NAC release. This difference demonstrates that L-cysteine efflux is a carrier-mediated process in contrast to NAC.

The efflux of amino acids is usually investigated in cells, and in the small intestine. These cells take up the ingested amino acids from the intestinal lumen and efflux them into the circulation. It has also been shown that *Escherichia coli* (*E. coli*) cells efflux cysteine from the cytoplasm to the periplasm by CydDC transporters to provide a balance in redox status.[22] Mutants in cydDC have been shown to cause a disturbance in redox status in the periplasm. In addition, lysosomes have been shown to have a system that is used to efflux the amino acids derived from enzymaticaly digested proteins.[23] This system, called LYAAT-1, is an amino acid transporter involved in the efflux of L-proline, L-alanine, or glycine from the organelle lumen to the cytosol. Similar or the same efflux systems may also function in erythrocytes. In these respects, the L-cysteine uptake and efflux processes in erythrocytes may have different functions. One function may be the modulation of the L-cysteine concentration in the plasma by lowering it when high and by increasing it when low thus providing homeostasis in the blood. A similar function is displayed by intestinal cell membranes.[24] Glucose transporters that function on the apical membrane influx glucose from the intestinal lumen into intestinal cells when glucose is increased especially after a carbohydrate-rich meal. This process is reversed on the basolateral membrane of intestinal cells, and in this case GLUT2 exports the concentrated glucose from the intestinal cells to the plasma. The liver is also known to display a similar function influxing glucose when its concentration in the plasma is high and effluxing glucose when the concentration is low. This type of function in erythrocytes becomes significant when feeding with cysteine-rich diets. Cysteine supplementation has been used for different purposes in children and in adults.[25,26] Cysteine supplements or formulations of cysteine-rich diets have become popular since it has been understood that it is the rate-limiting amino acid in the GSH synthesis. GSH functions in the scavenging of free radicals that are known to participate in the development of several-life threatening diseases.[27] Thus another function may be related to redox regulation in erythrocytes and in the plasma. Efficient uptake and the subsequent efflux of L-cysteine by erythrocytes may also contribute to the redox status of the plasma. L-cysteine is known to be oxidized easily when it is outside cells. Thus formation of cystine from L-cysteine would be limited in the plasma due to high uptake. On the other hand plasma thiol levels have been shown to be implicated in vascular disease. It has been reported that reduced cysteine is correlated with the amount of reduced homocysteine preventing its oxidation to disulfide form.[28] This function was correlated with the total blood cysteine concentration. Thus it was concluded that reduced cysteine plays a role in the maintenance of plasma redox status. In this sense, it could be suggested that L-cysteine efflux plays a role in the maintenance of the proper redox status in the blood plasma. Another function may be the fast and safe transport of cysteine from sites where its concentration is higher to distal tissues. Thus erythrocytes may function, in addition to being hemoglobin bags, in the transport of several other metabolites in addition to L-cysteine in the plasma.

REFERENCES

1) Griffith, O. W. (1999) Biologic and pharmacological regulation of mammalian glutathione synthesis. *Free Radic. Biol. Med.*, **27**, 922–935.
2) Gille, G. and Sigler, K. (1995) Oxidative stress and living cells. *Folia Microbiol.* (Praha), **40**, 131–152.
3) Clemens, M. R. and Waller, H. D. (1987) Lipid peroxidation in erythrocytes. *Chem. Phys. Lipids*, **45**, 251–268.
4) Eckert, K. G. and Eyer, P. (1986) Formation and transport of xenobiotic glutathione-S-conjugates in red cells. *Biochem. Pharmacol.*, **35**, 325–329.
5) Awasthi, Y. C., Garg, H. S., Dao, D. D., Partridge, C. A. and Srivastava, S. K. (1981) Enzymatic conjugation of erythrocyte glutathione with 1-chloro-2,4-dinitrobenzene: the fate of glutathione conjugate in erythrocytes and the effect of glutathione depletion on hemoglobin. *Blood*, **58**, 733–738.
6) Ansari, G. A. S., Singh, S. V., Gan, J. C. and Awasthi, Y. C. (1987) Human erythrocyte glutathione S-transferase: a possible marker of chemical exposure. *Toxicol. Lett.*, **37**, 57–62.
7) Strange, R. C., Jones, P. W. and Fryer, A. A. (2000) Glutathione S-transferase: genetics and role in toxicology. *Toxicol. Lett.*, **112**, 357–363.

8) Olive, C. and Board, P. (1994) Glutathione S-conjugate transport by cultured human cells. *Biochim. Biophys. Acta*, **1224**, 264–268.
9) Matsuda, Y., Epstein, L. F., Gatmaitan, Z. and Arias, I. M. (1996) The role of thiols in ATP-dependent transport of S-(2,4-dinitrophenyl) glutathione by rat liver plasma membrane vesicles. *Biochim. Biophys. Acta*, **1279**, 35–42.
10) Keppler, D. (1999) Export pumps for glutathione S-conjugates. *Free Radic. Biol. Med.*, **27**, 985–991.
11) LaBelle, E. F., Singh, S. V., Srivastava, S. K. and Awasthi, Y. C. (1986) Dinitrophenyl glutathione efflux from human erythrocytes is primary active ATP-dependent transport. *Biochem. J.*, **238**, 443–449.
12) Lunn, G., Dale, G. L. and Beutler, E. (1979) Transport accounts for glutathione turnover in human erythrocytes. *Blood*, **54**, 238–244.
13) Lu, S. C., Sun, W. M., Yi, J., Ookhtens, M., Sze, G. and Kaplowitz, N. (1996) Role of two recently cloned rat liver GSH transporters in the ubiquitous transport of GSH in mammalian cells. *J. Clin. Invest.*, **97**, 1488–1496.
14) Young, J. D., Wolowyk, M. W., Jones, S. M. and Ellory, J. C. (1983) Red-cell amino acid transport. Evidence for the presence of system ASC in mature human red blood cells. *Biochem. J.*, **216**, 349–357.
15) Awasthi, Y. C., Mısra, G., Rassın, D. K. and Srivastava, S. K. (1983) Detoxification of xenobiotics by glutathione S-transferase in erythrocytes: the transport of the conjugate of glutathione and 1-chloro-2,4-dinitrobenzene. *Br. J. Haematol.*, **55**, 419–425.
16) Sedlak, J. and Lindsay, R. H. (1968) Determination of sulfhydriyl groups in biological samples. *Anal. Biochem.*, **25**, 192–205.
17) Suliman, M. E., Barany, P., Filho, J. C. D., Qureshi, A. R., Stenvinkel, P., Heimburger, O., Anderstam, B., Lindholm, B. and Bergstrom, J. (2002) Influence of nutritional status on plasma and erythrocyte sulphur amino acids, sulph-hydryls, and inorganic sulphate in end-stage renal disease. *Nephrol. Dial. Transplant.*, **17**, 1050–1056.
18) Wang, F. X. and Cynader, M. X. (2001) Pyruvate released by astrocytes protects neurons from copper-catalyzed cysteine neurotoxicity. *J. Neurosci.*, **21**, 3322–3331.
19) Palacin, M., Estevez, R., Bertran, J. and Zorzano, A. (1998) Molecular biology of mammalian plasma membrane amino acid transporters. *Physiol. Rev.*, **78**, 969–1054.
20) Rosenberg, R. (1982) Na-independent and Na-dependent transport of neutral amino acids in the human red blood cells. *Acta Physiol. Scand.*, **116**, 321–330.
21) Yidiz, D. and Bagdadioglu, T. (2004) L-cysteine uptake is stimulated by 1-chloro-2,4-dinitrobenzene in vitro in human erythrocytes. *Toxicol. Mech. Methods*, **14**, 241–245.
22) Pittman, M. S., Corker, H., Wu, G., Binet, M. B., Moir, A. J. and Poole, R. K. (2002) Cysteine is exported from the Escherichia coli cytoplasm by CydDC, an ATP-binding cassette-type transporter required for cytochrome assembly. *J. Biol. Chem.*, **277**, 49841–49849.
23) Sagne, C., Agulhon, C., Ravassard, P., Darmon, M., Hamon, M., Mestikwy, S. E., Gasnier, B. and Giros, B. (2001) Identification and characterization of lysosomal transporter for small neutral amino acids. *PNAS.*, **98**, 7206–7211.
24) Berk, L., Matsudaira, Z. and Darnell, B. (2000) Transport across epithella. In *Molecular Cell Biology* (Tenney, S., Ed.), W. H. Freeman and Company, New York, pp. 597–604.
25) Badaloo, A., Reid, M., Forrester, T., Heird, W. C. and Jahoor, F. (2002) Cysteine supplementation improves the erythrocyte glutathione synthesis rate in children with severe edematous malnutrition. *Am. J. Clin. Nutr.*, **76**, 646–652.
26) Sen, C. K. and Packer, L. (2000) Thiol homeostasis and supplements in physical exercise. *Am. J. Clin. Nutr.*, **72**, 653S–669S.
27) Lomaestro, B. M. and Malone, M. (1995) Glutathione in health and disease: pharmacotherapeutices. *Ann. Pharmacother.*, **29**, 1263–1273.
28) Andersson, A., Lindgren, A., Arnadottir, M., Prytz, H. and Hulfberg, B. (1999) Thiols as a measure of plasma redox status in healthy subjects and in patients with renal or liver failure. *Clin. Chem.*, **45**, 1084–1087.